DOI:
10.1097/JCP.0000000000000715

Document Version
Peer reviewed version

Clozapine associated agranulocytosis -treatment with G-CSF/GM-CSF, a systematic review Running title: G-CSF/ GM-CSF for clozapine agranulocytosis

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Word count:

Abstract: 198

Article: 2907

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Acknowledgment and disclosures:

Only 2 authors (DT, FG) have a potential conflict of interest, although not in relation to this work.

All other authors (JL, SM, EW, AK, RJF, AM and JHM) declare no conflict of interest

DT has the following declaration of interest. Advisory Board member for: Lundbeck, Servier, Sunovion; lectures for Janssen, Lundbeck, Otsuka, Servier: research funding from: BMS, Janssen, Lundbeck.. FG has received honoraria for advisory work and lectures from Roche, BMS, Lundbeck, and Sunovion and has a family member with professional links to and share options with Lilly and GSK.

The other authors have no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; there are no other relationships or activities that could appear to have influenced the submitted work.

This paper represents independent research part funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Abstract

Purpose/Background

Clozapine is associated with haematological abnormalities, notably neutropenia, which may progress to agranulocytosis. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been used to reduce the frequency and duration of

clozapine-associated neutropenia. This review aims to explore the use, efficacy, and tolerability of these cytokines in the treatment of clozapine-associated agranulocytosis.

Methods/Procedures

We conducted a systematic review of published interventional, observational studies, case series, and case reports where G-CSF/GM-CSF was used to treat clozapine-associated agranulocytosis.

Findings/Results

We identified 29 reports (40 patients). The median duration of neutrophil recovery time after stopping clozapine and starting cytokine treatment was 7.0 days (range 2-13 days) for those with agranulocytosis (absolute neutrophil count (ANC) < 0.5 x 10^9 cells/L). Ninety-four percent (n=29) had no serious adverse reactions, and no deaths occurred.

Implications/Conclusions

Our findings indicate that G-CSF/GM-CSF use is well tolerated, and suggest that GCSF can sometimes be safely used to reduce the duration of neutropenia associated with clozapine use. However, the interpretation of this outcome is difficult given the likely publication bias for positive outcomes in case reports.

Keywords: granulocyte colony stimulating factors; G-CSF; GM-CSF; treatment-resistant; schizophrenia; clozapine

Introduction

Clozapine remains the gold-standard treatment for treatment resistant schizophrenia. (1, 2) However, the use of clozapine is restricted in part due to its risk of inducing neutropenia, which may progress to agranulocytosis unless clozapine is promptly withdrawn. The cumulative incidence of clozapine-induced neutropenia (defined as an absolute neutrophil count (ANC) < 1.5 x 10^9 cells/L) is 2.7 % over the first year of use. (3) The cumulative incidence of clozapine-induced agranulocytosis (CIA) (defined as an ANC < 0.5 x 10^9cells/L) is 0.8 % at 1 year and 0.91 % at 18 months, (4) with the highest incidence at 6-18 weeks after commencing clozapine. (5) Clozapine use is accompanied by

the requirement for regular full blood count (FBC) monitoring in many countries, and if the total leucocyte and/or neutrophil counts indicate the development of neutropenia or agranulocytosis, clear criteria exist for drug withdrawal. The strategy combined with modern treatment options has largely prevented deaths from this serious adverse reaction: with the mortality rate from CIA is estimated to be 0.01–0.03 % (case-fatality rate 2.2–4.2 %). (6)

In most countries, (7) when neutropenia occurs during clozapine treatment, clozapine must be discontinued. Depending on a patient's clinical state, management may be supportive with daily monitoring of the full blood count until the neutrophil count normalizes, or may involve the administration of antibiotics if there is a febrile neutropenia. Granulocyte colony-stimulating factors (G-CSFs), and granulocyte-macrophage colony-stimulating factors (GM-CSFs) have been used to treat clozapine associated neutropenia. Both agents are typically administered during chemotherapy in order to reduce the incidence or the duration of neutropenia. (8, 9) They stimulate proliferation and differentiation of committed myeloid progenitor cells in the bone marrow.(10) G-CSF has supplanted the use of GM-CSF in recent years. While both agents stimulate granulocyte production, G-CSF also shortens the maturation phase from progenitor cells to the neutrophil granulocyte, further increasing the peripheral neutrophil count.(11) Filgrastim and lenograstim are the most commonly used G-CSFs, and are administered by subcutaneous injection. They both have short plasma half-lives (lenograstim $t_{1/2}$ approximately 3 hours(12) ; filgrastim $t_{1/2}$ approximately 3.5 hours, (13) and are excreted renally.

Both G-CSF and GM-CSF have been used increasingly to treat clozapine induced agranulocytosis.(14, 15) However, knowledge of their use in clozapine patients is limited. Their use in the treatment of agranulocytosis induced by non-chemotherapeutic agents has been reviewed, (16) however no similar review of their use for clozapine associated agranulocytosis has been performed.

Aims

We aimed to review the literature to investigate the effect of G-CSFs and GM-CSFs in reducing the duration of clozapine associated agranulocytosis; to describe the doses of G-CSF used and; to review the tolerability of these agents.

Methods

We performed a literature search to identify peer-reviewed interventional and observational studies, case series and case reports, up until August 2016, investigating or describing G-CSF or GM-CSF as a treatment for clozapine associated agranulocytosis. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standard. (17)

Inclusion criteria

Studies and case reports of patients (no age restrictions) who were treated with G-CSF or GM-CSF for clozapine associated agranulocytosis (with an ANC < 0.5 $x10^9$ cells/L), including patients who developed a neutropenia during rechallenge. For those cases of neutropenia with an ANC < 0.1 $x10^9$ cells/L, we described it as severe agranulocytosis, to distinguish a condition where an individual is at an increased risk of morbidity and mortality from infections, and to make it distinct from agranulocytosis (ANC< 0.5 $x10^9$ cells/L).(18)

Exclusion criteria

Studies were excluded if: G-CSF or GM-CSF was used to support the maintenance of clozapine treatment during chemotherapy; if there was insufficient laboratory data to permit further evaluation of the report; they described a neutropenia with an absolute neutrophil count (ANC) > 0.5 $x10^9$ cells/L or; with an ANC of >1.5$x10^9$ cells/L.

Information sources and searches

Two independent reviewers (JL and SM) performed an electronic search using PubMed, Medline, Scopus, EMBASE and Google Scholar from inception until August 2016. The following search terms were used, alone and in combination: Granulocyte Colony-Stimulating Factor OR granulocyte-macrophage colony-stimulating OR GCSF OR G-CSF OR GMCSF OR GM-CSF AND clozapine OR clozaril OR denzapine OR zaponex OR leponex. In addition, the reference lists of the retrieved articles and relevant review articles were examined for further reports

Study selection and exclusion

All extracted reports were examined independently by two authors (JL and SM) and a list of full text articles established. Authors were contacted for clarification where necessary. There were 29 qualifying reports, 7 of which were case series and 22 case reports. In total 40 patients were identified who received G-CSF or GM-CSF as treatment for clozapine associated agranulocytosis.

Primary and secondary outcomes

The primary outcome was the time for neutrophil recovery after starting G-CSF (as defined by recovery either to within the normal range of a local laboratory, or a neutrophil count > 2.0 $x10^9$ cells/L). For secondary outcomes, we examined for associations between neutrophil recovery time and the nadir neutrophil count (specifically the occurrence of agranulocytosis), the type of G-CSF or GM-CSF used, and the cumulative dose of G-CSF or GM-CSF used. The tolerability of G-CSF and GM-CSF was assessed, and outcomes including mortality due to complications secondary to neutropenia or agranulocytosis were recorded. The incidence of rebound leukocytosis (as defined by each study or a WCC>10.0 $X10^9$/L) was recorded and associations between leukocytosis and dose and duration of G-CSF or GM-CSF use were assessed.

Data extraction

The following information was extracted where possible: demographic and clinical characteristics of patients, mean clozapine dosage (mg/d) at the time of the neutropenic event, and duration of clozapine therapy prior to onset of neutropenia , plasma clozapine concentrations reported pre neutropenia, nadir neutrophil count, duration of neutropenia prior to the use of G-CSF or GM-CSF, duration of neutropenia following administration of G-CSF or GM-CSF and duration of G-CSF or GM-CSF use and total duration of neutropenia; antibiotic use; adverse effects associated with G-CSF or GM-CSF and; rebound leukocytosis associated with G-CSF or GM-CSF use.

Results

Study selection, study and participant characteristics

The study selection process, search results, and reasons for exclusion are given in figure 1.

The initial search yielded 766 references. After checking titles and abstracts, 57 full texts were screened and 29 of these (40 patients) were included for data extraction. (15, 19-46) All were case series or reports; no interventional or observational studies were identified.

Acute treatment with G-CSF for clozapine-associated neutropenia or agranulocytosis

The demographic and clinical characteristics of the group are shown in table 1. Twenty-seven (67.5% of total population) had a severe agranulocytosis, with an ANC <0.1 $x10^9$ cells/L. Nine patients (39.0% of those in whom a clozapine first treatment or rechallenge status was reported) had developed a further neutropenia during clozapine rechallenge. There was insufficient data in relation to plasma clozapine concentrations to report in this review.

Clozapine was discontinued in all patients at onset of neutropenia. Antibiotics were administered to 21 patients. Bone marrow aspiration was performed in 11 patients (55.0% of those cases in which bone narrow aspiration was reported to be performed or not). Twenty-three patients (97.5% of those in whom G-CSF type was specified) were treated with filgrastim, while a single patient was treated with sarcograstim. The outcome characteristics of G-CSF or GM-CSF use are described in table 2. There were no deaths recorded.

The median time to neutrophil recovery was 7.0 days for all patients. For those with an ANC of 0.1-0.5 $x10^9$ cells/L, the median duration of neutrophil recovery time was 6.0 days. The mean total dose of filgrastim used was 399 ± 198 mcg (dose range 125-900 mcg). There was no significant difference in mean time to neutrophil recovery between those treated with a lower daily dose of filgrastim (i.e. 5 mcg/kg/day or less) (n=4) (7.0±1.4 days) compared to those treated with 10mcg/kg/day (n=3) (6.3 ±3.5 days) (t=0.352, p=0.739). The dose of filgrastrim used was not significantly correlated with the time to neutrophil recovery (r=0.554, p=0.050) following its administration controlling for concurrent antibiotic use, baseline neutrophil count and duration of neutropenia prior to G-CSF administration.

Adverse events

No adverse reactions to G-CSF or GM-CSF were reported in 29 cases (93.5% of those in whom reports referred to the presence or absence of adverse events). One female patient developed a thrombocytosis with clinical signs of deep vein thrombosis (DVT)(43) after seven days treatment with G-CSF at a dose of 300 mcg/day(with a maximum platelet count of 800 x 10^9/L). The platelet count normalised seven days after the discontinuation of G-CSF, with no further evidence of thrombosis.(43) Another patient,(42) a 30 year old man, developed a right middle cerebral artery infarct, after three days of treatment with GM-CSF 300 mcg/day (ANC= 0.1 x10^9 cells/L).(42) The activated partial thromboplastin time was prolonged at 39 s (normal range < 36 s), with a normal prothrombin time. There were no reports of bony or musculoskeletal pain, fever, headache, arthritic flare ups or splenomegaly. No deaths occurred.

Ten (46% of those cases in whom WCC were reported following G-CSF or GM-CSF use) of the patients had evidence of a rebound leucocytosis. For 11 of these cases, the average time to normalisation of the leucocytosis (as defined by each study or a WCC< 10.0 X10^9/L) was 11.6 (15.5) days (range 0-50 days). No adverse events were reported in association with these events. The average dose of filgrastim used was not significantly associated with the occurrence of leucocytosis (mean dose 540.0 ±270.6mcg in those with a leucocytosis (n=7) compared to a mean dose of 411.4 ±144.6 mcg in those with no leucocytosis (n=7) (t=1.109, p=0.289)). Further, the average duration of G-CSF use was not significantly associated with the occurrence of leucocytosis (mean of 7.9 ±3.0 days in those with leucocytosis compared to a mean of 7.3 ±3.1 days in those with no leucocytosis (t=0.434, p=0.669).

Discussion

In this, the first systematic review of the use of G-CSF for the treatment of clozapine associated neutropenia and agranulocytosis, we identify that G-CSF treatment may be beneficial for patients in aiding with neutrophil recovery following the discontinuation of clozapine.

GCSF for acute treatment of clozapine associated agranulocytosis and neutropenia

The median time to neutrophil recovery in agranulocytosis following administration of G-CSF was 7.0 days. This is shorter than the 12 day median duration of neutropenia recovery associated with clozapine use without treatment with GCSF identified in a systematic review of case reports of drug induced agranulocytosis (defined by ANC<$0.5x10^9$) (for case reports published since 1990).(16) Thus, the use of G-CSF in clozapine associated neutropenia may shorten the duration of neutropenia by half, and is notably less than the previously reported duration of clozapine associated agranulocytosis (defined by ANC<$0.5x10^9$) not treated with G-CSF of 14-21 days.(15) Given that the duration of agranulocytosis is an important prognostic factor in drug induced agranulocytosis,(47) our finding of a duration of neutrophil recovery with G-CSF use of 7 days, is clinically significant and suggests that G-CSF use should be more prominently considered for those with clozapine associated agranulocytosis.

For those with severe agranulocytosis (ANC <$0.1x10^9$ cells/L), the mean time to neutrophil recovery following administration of G-CSF was 7.7 ± 3.1 days, and is comparable to the recovery time identified in a systematic review of case reports in which G-CSF was used to treat non-cytotoxic drug induced agranulocytosis (all drugs and not just clozapine) (defined as an ANC<$0.1\ x10^9$ cells/L) (mean=7.7±5.1 days (n=100 cases).(16) Our findings for an enhanced neutrophil recovery time with the use of G-CSF in severe agranulocytosis, mirrors those for its use in agranulocytosis and severe agranulocytosis caused by other non-chemotherapeutic drugs, and supporting the use of G-CSF for this clozapine associated agranulocytosis.

No deaths were reported in those treated with G-CSF. G-CSF or GM-CSF was well tolerated in all but two cases, (42, 43) with no severe adverse events identified in the others. In one case, GM-CSF was associated with arterial thrombosis. This case was associated with a prolonged APTT, which may be seen with antiphospholipid syndrome-though the patient was negative for anticardiolipin antibodies. A previous meta-analysis suggested that the use of GM-CSF compared to G-CSF was associated with an increased thrombotic risk, (48) though we were unable to investigate this due to the low numbers treated with GM-CSF. In the other case with an adverse reaction, a female patient treated with G-CSF developed a DVT. (43)

Forty-six percent of patients had a rebound leucocytosis, following the administration of G-CSF for the treatment of clozapine associated neutropenia, which lasted for an average duration of 12 days. This was not associated with the use of a higher mean dose of filgrastim or with a longer duration of G-CSF use. No overt clinical events were associated with this, including no reports of thrombotic events. However, given the high rate of occurrence of rebound leucocytosis, we recommend that clinicians continue to monitor white cell and platelet counts until normalisation occurs and monitor patients for clinical evidence of venous thrombosis; and for arterial thrombosis, monitoring for cardiac symptoms such as chest pain, neurological symptoms such as weakness, speech disturbance, confusion, and pain in limbs, and for pulmonary oedema, and hyperviscosity syndrome (e.g. headache, blurred vision).

Limitations

The primary limitation is the possibility of publication bias. Most patients with clozapine associated neutropenia/agranulocytosis are not reported in the literature, and it is likely that G-CSF is more widely used for the treatment of these episodes than is indicated here. It is possible that a bias towards reporting cases in which there is a favourable outcome after treatment with G-CSF may have occurred. It is also possible that cases in which adverse reactions or deaths have occurred are more likely to have been reported. There is no agreed method to assess the effect of publication bias in case reports. Further caution is required when interpreting the results, as data is lacking on numerous confounding factors which may have influenced when and why the decision was made to intervene with cytokine treatment, factors that are treated differently from one case to the next. Finally, as no comparative or observational studies were identified, we are unable to confirm that the duration of time to recovery is shortened, or that morbidity/mortality are reduced. The possibility remains that no treatment -- discontinuing clozapine and allowing recovery to take its course -- may be the most beneficial approach.

Conclusions

Our review demonstrates benefits for the use of G-CSF for patients with clozapine associated neutropenia/agranulocytosis. A prospective placebo controlled trial to establish the efficacy of G-CSF in clozapine associated neutropenia/agranulocytosis would be the gold standard study. The rarity of clozapine-induced neutropenia may make such a study impractical outside highly specialised centres. As such, observational and retrospective studies to establish the efficacy of G-CSF in clozapine associated agranulocytosis would be the first choice for future research. Until such studies are conducted, this review of the available evidence suggests that G-CSF may be a valuable tool in the treatment of clozapine associated neutropenia. However, the possibility of publication bias towards favourable outcomes cannot be ruled out.

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Table 1. Characteristics of Patients with clozapine associated agranulocytosis (n=40)

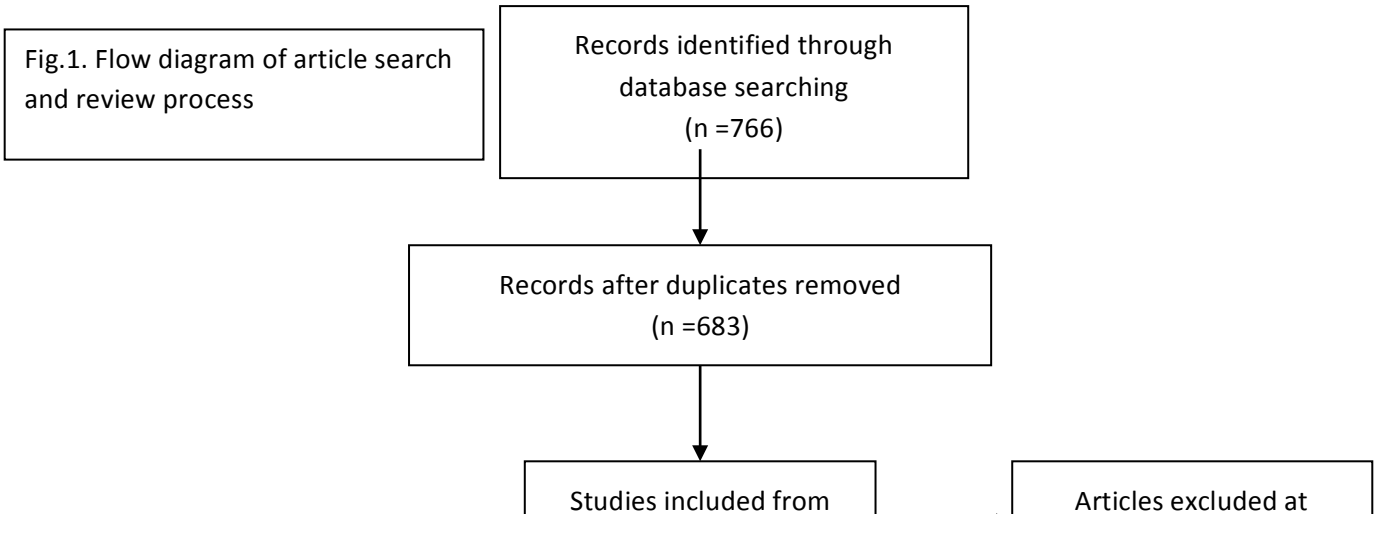
Gender n (%) Male Female Not reported	 21 (52%) 15 (38%) 4 (10%)
Mean age ± SD (range)	45.2 ±14.4 (13-85)
Clozapine rechallenge Yes No Not reported	 9 (22.5%) 14 (35%) 17 (42.5%)
Mean clozapine dose at time of neutropenia (range) mg	393.1 ±18.6 (25.0-900.0)
Mean duration of clozapine use prior to neutropenia onset± SD (median; range) days	195.2±336.0 (56; 27-1620)

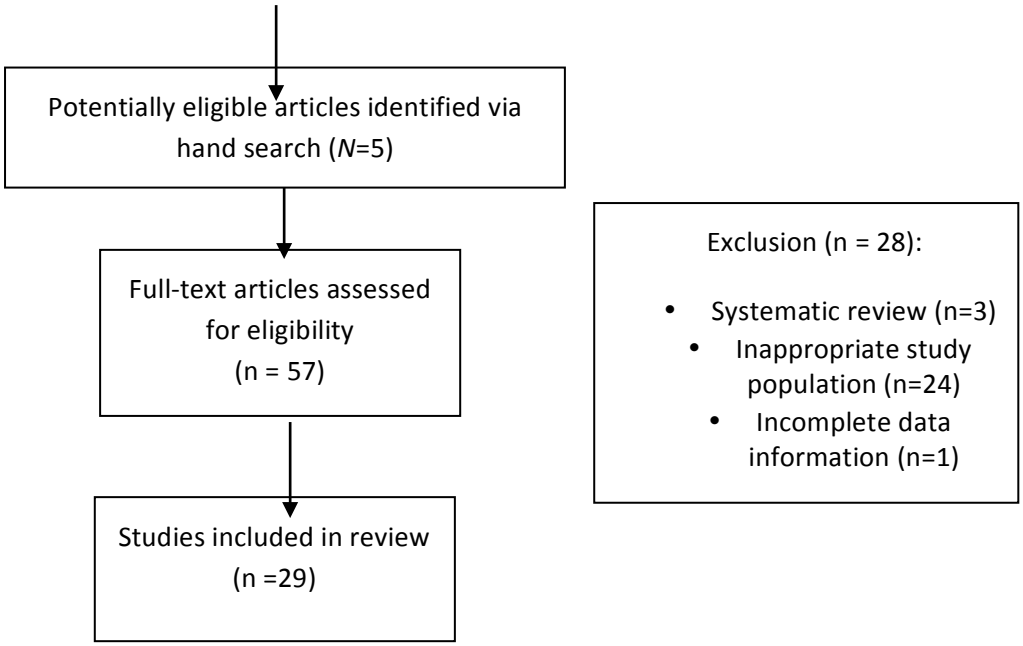
Mean neutrophil count nadir ± SD (range) $x10^9$ cells/L	0.11 ±0.14 (0-0.5)
Severe agranulocytosis (ANC <1.5 $x10^9$ cells/L) Yes No	 27 (67.5%) 13 (32.5%)
Antibiotics used Yes No Not reported	 21 (53%) 11 (27%) 8 (20%)
G-CSF use GM-CSF use Both G-CSF and GM-CSF use	36 (90%) 3 (7.5%) 1 (2.5%)
Adverse events Yes No Not reported	 2 (5.0%) 29 (72.5%) 9 (22.5%)
Rebound leucocytosis Yes No Not reported	 10 (25%) 12 (30%) 18 (45%)

Table 2 Outcome and response to G-CSF and GM-CSF in clozapine associated agranulocytosis (n=40)

	All patients	Agranulocytosis (ANC 0.1-0.5 X109 cells/L)	Severe agranulocytosis (ANC <0.1X109 cells/L)	T test; p value
Recovery	40	13	27	
Mean duration of neutropenia prior to use of G-CSF (±SD) **(median; range) days**	4.1 ±4.4 (2.5;0-17)	3.5±4.6	4.3±4.3	0.472; 0.641
Mean duration of neutrophil recovery after G-CSF initiation	7.2± 3.0 (7.0; 2-	5.9±2.6	7.7±3.1	1.660;

(±SD) **(median; range) days**	13)			0.107
Mean total duration of neutropenia (pre and post G-CSF) (±SD) **(median; range) days**	11.6± 4.7 (11;3-26)	10.4±4.6	12.2±4.7	1.058; 0.298




Potentially eligible articles identified via hand search (*N*=5)
Full-text articles assessed for eligibility (n = 57)
Studies included in review (n =29)
Exclusion (n = 28):
• Systematic review (n=3)
• Inappropriate study population (n=24)
• Incomplete data information (n=1)